Summer 2005

Investigating signaling and metabolism of PLC- and PLD-derived phosphatidic acid in the root apical meristem of Arabidopsis thaliana

Emily Vaughn

Investigating signaling and metabolism of PLC- and PLD-derived phosphatidic acid in the root apical meristem of *Arabidopsis thaliana*

Emily Anne Vaughn, Alfredo Cruz Ramírez, Luís Herrera Estrella
Departamento de Ingeniería Genética, Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional, Irapuato, Guanajuato, México
HHMI International Summer Research Fellow, 2005

Abstract

Our experiment examined the role that two different phospholipases play in the development of *Arabidopsis thaliana* root apical meristem architecture. The phospholipases (PLC and PLD) both synthesize phosphatidic acid (PA), a lipid second messenger, but go about it differently. We were interested in whether the PA derived from either PLC or PLD is more important for proper meristem development. We observed that certain PLC and certain PLD treatments make a difference in transcription factor levels (and thus cell identity) and root architecture. However, certain doubts about the specificity of the treatments mean that we cannot necessarily attribute our observations to our proposed pathway. The data collected suggest many interesting future projects.

Introduction

The principal focus of the Herrera lab (Departamento de Ingeniería Genética, CINVESTAV-IPN) in Irapuato, Mexico, is how plants acquire phosphorus and use it efficiently. The lab uses *Arabidopsis thaliana* mutants and marker lines to study the physiological mechanisms by which plants obtain sufficient phosphorus (P) when P is scarce.

Interestingly, the abundance of phospholipids decreases during P-starvation, while the abundance of other lipids increases. We hypothesize that phospholipids are broken down to phosphatidic acid (PA) during P-starvation to make phosphorus available for metabolic functions, and/or to trigger stress-response processes.

PA can be derived from the phospholipase C (PLC) or phospholipase D (PLD) pathway (Fig 1, Fig 2). Our project examines the effect of manipulating PA levels synthesized by different pathways on a group of mitotically inactive cells in the root apical meristem, called the quiescent center (QC) (Fig 5).

Since this was a "fishing" experiment, rather than predicting which pathway will prove more important in conferring cell identity and organization, we hoped to disprove the null hypothesis: that the pathways are equally important.

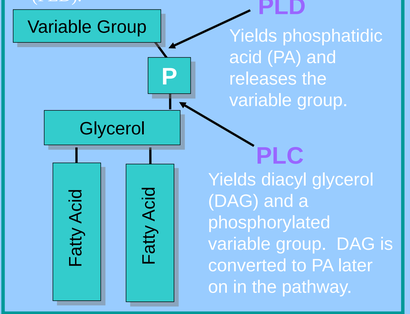
Central Questions

- What adaptive responses occur in the root meristem as a result of changes in PA biosynthesis, specifically in the quiescent center (QC)?
- Is the PA that functions as a lipid secondary messenger to the QC primarily derived from the PLC or the PLD pathway?

Who Cares?

- Findings will contribute to the general understanding of root meristem metabolism and function, and the effects of P-starvation on *Arabidopsis* roots.
- Results may be useful for the creation of genetically engineered crops.

Fig 1. Diagram of enzymatic action sites of phospholipase C (PLC) and phospholipase D (PLD).



Methods

We exposed 5-7 day-old *Arabidopsis* seedlings to four different treatments (Fig 2) for 24 hours to visualize the differential effects of manipulating aspects of the two routes of PA synthesis. Effects were assessed based on QC identity, general root architecture (Fig 5), and expression of a transcription factor marker called GUS. We used two promoter trap lines to stain specific regions of the root with GUS: p*PLT1*:GUS and p*QC46*:GUS. The PLT protein is expressed throughout the root apical meristem, most densely in the QC and columnella. QC46 is a specific identity marker of the QC, whose gene function is unknown.

Five to ten plants were exposed to each concentration of each treatment. At present, only the Neomycin experiment has been replicated

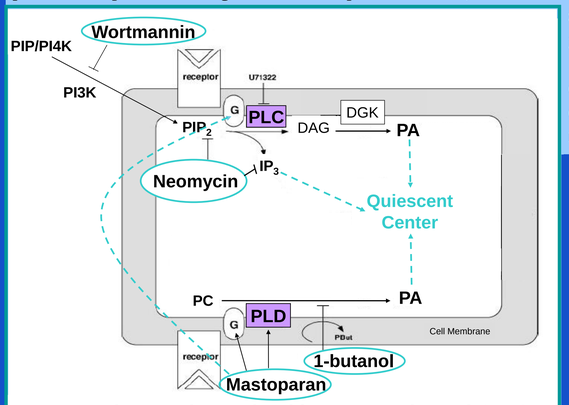


Fig 2. PA production pathways, altered from Meijer and Munnik (2003). Dotted lines indicate interactions proposed as a result of these experiments. Turquoise ovals indicate our treatments.

References

Aida, M.,Beis, D., Heidstra, R., Willemsen, V., Noh, Y.-S., Amasino, R., Sheres, B. (2004) The *PLETHORA* genes mediate patterning of the *Arabidopsis* root stem cell niche. Cell 119, 109-120.

Dhonukshe, P., Laxalt, A.M., Goedhart. J., Gadella, T.W., Munnik, T. (2003) Phospholipase D activation correlates with microtubule reorganization in living plant cells. Plant Cell 15: 2666–2679.

Legendre, L., Rueter, S., Heinstein, P.F., Low, P.S. (1993) Characterization of the oligogalacturonide-induced oxidative burst in cultured soybean *(Glycine max)* cells. Plant Physiol 102: 233-240.

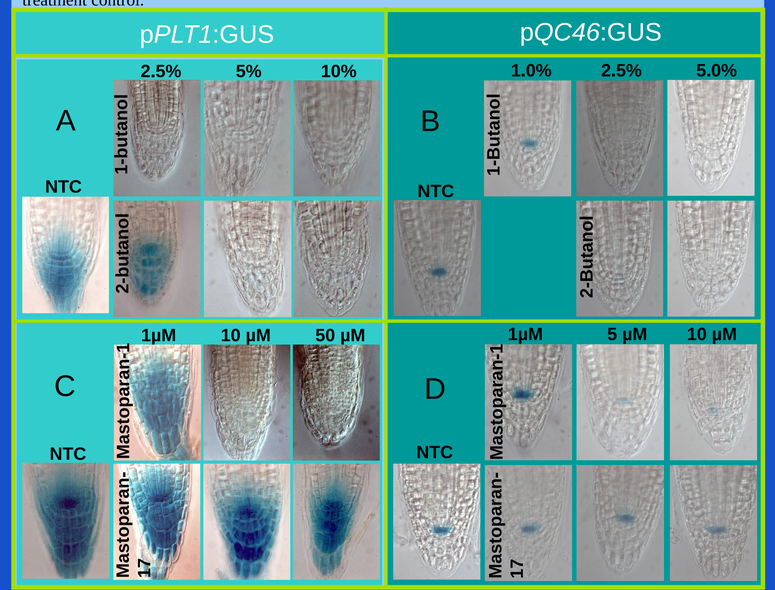
Meijer, H.J.G., and Munnik, T. (2003). Phospholipid-based signaling in plants. Annu. Rev. Plant Biol. 54, 265–306.

Scheres, B., Benfey, P., Dolan, L. (2002) Root development. The Arabidopsis Book.

Van den Berg, C., Willemsen, V., Hendriks, G., Weisbeek, P. Scheres, B. (1997) Short-range control of cell differentiation in the *Arabidopsis* root meristem. Nature 390, 287-289.

PLD Pathway

Fig 4. Results of treatment with various **PLD** pathway-manipulating reagents at different concentrations. Tissue was clarified, whole-mount visualized on slides in 50% glucose, and observed at 1000X. NTC: no treatment control.

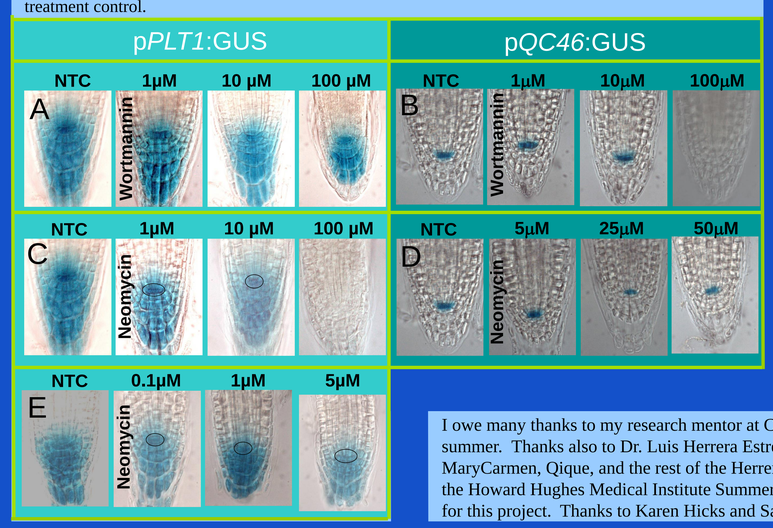


Results

- **Butanol:** GUS expression in1-butanol-treated roots was greatly reduced at even small concentrations, yet roots did not display disrupted organization in either marker line (Fig 3. A, B). Concentrations above 2.5% are not reliable, as changes were observed in the 2-butanol control.
- **Mastoparan:** Decreases in GUS expression were observed at the same concentration in both marker lines, suggesting that MP-1 similarly impacts the transcription factors of both lines (Fig 3. C, D). Treatment with high concentrations resulted in a compressed meristem phenotype. **In some roots, we observed loss of QC identity, and saw GUS expression in initial cells.**
- **Wortmannin:** There was a more pronounced decrease in GUS expression in p*QC46*:GUS than in p*PLT1*:GUS (Fig 4. A, B). No disruption of meristem architecture was observed.
- **Neomycin:** A compressed meristem phenotype was observed in p*QC46*:GUS at lower concentrations than decreased GUS expression (Fig 4. C, D). **In p*PLT1*:GUS we observed division of QC cells** (Fig 4. C, ovals)**.** This experiment was replicated with a finer concentration gradient and division of QC cells was again observed (Fig 4. E).

PLC Pathway

Fig 5. Results of treatment with various **PLC** pathway-manipulating reagents at different concentrations. Tissue was clarified, whole-mount visualized on slides in 50% glucose, and observed at 1000X. NTC: no treatment control.



Discussion

- **Neomycin induces QC cells to divide.** QC cells are mitotically inactive; they do not normally divide. This result inspired us to investigate the ways in which Neomycin might induce cells to move from G_0 to G_1. Neomycin may influence cell cycle transitions by negatively regulating IP_3, a molecule that is active in many cell cycle processes.
- **GUS is expressed in the cortex/epidermal initials in p*QC46*:GUS.** *QC46* transcription factors are only found in QC cells: the presence of GUS in initials, and not in the QC, suggests that QC identity was transferred to initials. This is unexpected; QC cell loss typically results in differentiation of cell lines (van den Berg et al., 1997).
- **Diminished expression of GUS in the QC implies loss of QC identity, but not necessarily change in architecture.** The QC is essential for conferring identity to surrounding cells, thereby regulating general root architecture. However, sometimes we observed roots that showed decreased GUS expression, but normal root architecture.
- **Treatments are non-specific.** Most of the treatments have a variety of effects on *Arabidopsis*. For example, Dhonukshe et al. (2003) propose a relationship between PLD and the cytoskeleton. Therefore, our observations are not necessarily the result of manipulation of our the PLC and PLD pathways. While we have not fully answered our original questions, through these experiments we have synthesized better questions, and generated ideas for future experiments.
- **Concentrations may not have been optimal.** The literature on several of the treatments report conflicting ideal maximum and minimum concentrations.
- **There may be cross-talk between the PLC and PLD pathways.** MP-1 (which we used as a PLD treatment) increases IP_3 (a product of the PLC pathway) levels in soy culture cells (Legendre et al., 1993). Also, there is evidence that MP-1 acts on all heterotrimeric G proteins, not only those that feed into the PLD pathway.

Root Apical Meristem Architecture

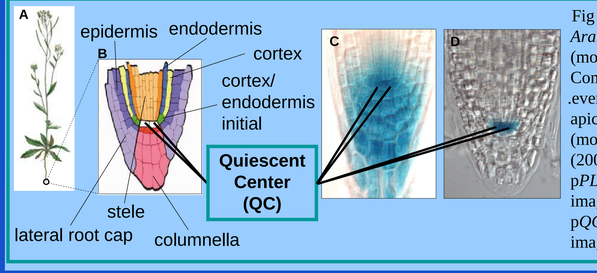


Fig 5. **A.** Whole, mature *Arabidopsis thaliana* (modified from Scientific Computing, http://scicomp.evergreen.edu/) **B.** Root apical meristem architecture (modified from Aida et al. (2004) **C.** Typical p*PLT1*:GUS meristem (our image) 1000X **D.** Typical p*QC46*:GUS meristem (our image) 1000X

Future Directions

- **Test confidence in Mastoparan.** We had assumed that MP-1 exclusively targets the PLD pathway, but there is some evidence that MP-1 non-specifically induces genes through heterotrimeric G proteins (Legendre et al., 1993). To test this, we will cross a heterotrimeric G protein loss-of-function mutant and *QC46*:GUS (experiment under way). If division of QC cells *is* observed in the absence of functional G proteins, then the MP-1 that induced the division is *not* introduced to the cell via G proteins. If division of QC cells is *not* observed, then the MP-1 *is* delivered by G proteins. The next step is to identify the downstream receptors: is PLC, PLD, or another route responsible?
- **Does Neomycin act by inhibiting Inositol 1,4,5 Phosphate (IP_3)?** We will treat *QC46*:GUS roots with Neomycin and IP_3. If the double-treatment restores the *QC46*:GUS phenotype, then the alteration was due to a decrease in IP_3 rather than a decrease in PLC-derived PA. If the results of the previous experiment find that MP-1 acts indiscriminately upon heterotrimeric G proteins, then we would expect treatment of *QC46*:GUS with IP_3 and MP-1 to yield similar phenotypes.

Acknowledgements

I owe many thanks to my research mentor at CINVESTAV, Dr. Alfredo Cruz Ramírez, for his guidance and support in and out of the lab througout the summer. Thanks also to Dr. Luis Herrera Estrella, Alejandra Chacón-López, Francisco Razo, Dr. Andrés Cruz Hernández, Anahí Pérez, Krishnia and MaryCarmen, Qique, and the rest of the Herrera lab and Irapuato crew for their various and invaluable contributions. Thank you to Dr. Joan Slonczewski, the Howard Hughes Medical Institute Summer Research Fellows program, and the Kenyon Biology Department for giving me the opportunity and funding for this project. Thanks to Karen Hicks and Sam Shopinski for assistance in the editing of this report and poster.